REMARKS

Claims 1-16, 18-27 and 32-43 are in this application. Claims 16-18, 21, 24 and 39-43 were examined. Claims 1-15, 19, 20, 22, 23, 25-27 and 32-38 were withdrawn. Claim 16 has been amended to include the subject matter of claim 17 "wherein the cardiac toxicity, myocardial ischemia, myocardial infarction or heart failure is induced by an anthracycline antineoplastic" and claim 17 has been cancelled. Support for this amendment is also found, inter alia, in paragraphs [0008] to [0014] of the published application. Claims 21, 24 and 43 have been cancelled.

The Examiner has rejected all the elected claims 16-18, 21, 24, and 39-43, as being obvious over Liu et al (Biochem. Cell Biol. Vol 78, 2000, 447-453) and Barone et al (US 5,405,863). This is respectfully traversed.

Three distinct types of anthracycline-induced cardiotoxicity have been reported. First, acute or subacute injury can occur immediately after treatment. This rare form of cardiotoxicity may cause transient arrhythmias, a pericarditis-myocarditis syndrome, or acute failure of the left ventricle. Second, anthracyclines can induce chronic cardiotoxicity resulting in cardiomyopathy. This is the more common form of damage and is clinically the most important. Finally, late-onset anthracycline cardiotoxicity causing late-onset ventricular dysfunction and arrhythmias, which manifest years to decades after anthracycline treatment has been completed, is increasingly recognized.

Although, the cause of anthracycline-induced cardiotoxicity is probably multifactorial, anthracyclines cause the selective inhibition of cardiac muscle gene expression for α-actin, troponin, myosin light-chain 2, and the M isoform of creatine kinase *in vivo*.

As reported by Suter et al (Annals of Oncology, **2002**,13:647-649), the prognosis of anthracycline-induced cardiotoxicity is poor, and possibly even worse than those of ischemic or idiopathic dilated cardiomyopathies.

Thus, in view of the ominous prognosis for patients with anthracycline-induced cardiomyopathy, the success with the current known therapies such as angiotensin-converting enzyme inhibitors, ß-blockers, antioxidants is limited.

The Examiner states that the claimed invention is obvious over the combination of Liu and Barone.

The Examiner states that Liu et al. have compared the effects of three pineal indoles, melatonin, 5-methoxytryptamine (5-MT), and 5-methoxytryptophol on the levels of reduced glutathione (GSH) and oxidized glutathione (GSSG), and the activities of the enzymes catalase, superoxide dismutase (SOD) and glutathione reductase in the rat liver, kidney and brain. The reported results of this study suggest that the activities of SOD and glutathione reductase in rat kidney and brain tissues were elevated by all three pineal indoles. Further melatonin and 5-methoxytryptamine, but not 5-methoxytryptophol, elevated glutathione reductase activity in the rat liver. The catalase activity in rat kidney tissue was elevated by all three pineal indoles.

Although, Liu et al. report the activation of antioxidant enzymes in the rat kidney, liver and brain by the three pineal indoles and suggest that pineal indoles may have free radical scavenging activities, no where in the article is it stated or suggested that the pineal indoles could be effective in treating the anthracycline induced cardiotoxicity.

Thus, Liu et al, in order to be available as full prior art with respect to the present invention, suffers from the inherent deficiency in that it fails to disclose that 5-methoxytryptamine, being an antioxidant or free radical scavenger that could be used to treat cardiac toxicity, myocardial ischemia, myocardial infarction or heart failure. On page 3 of the Office Action, the Examiner refers to this deficiency.

To remedy the abovementioned deficiency, the Examiner has suggested that Barone et al (US patent 5,405,863) disclose that antioxidants or oxygen free radical scavengers can protect the cardiovascular system and hence, overcomes the inherent deficiency of Liu et al. Applicants respectfully disagree.

Barone et al. is specifically related to hydroxycarbazole compounds and not to indole derivatives. Moreover, this patent discloses use of hydroxycarbazole compounds, as being oxygen radical scavengers or antioxidants for protection of the cardiovascular system from traumatic and post traumatic injury associated with myocardial infarction, as well as for prevention of oxidative tissue damage to organs afflicted with disease induced ischemic trauma and, therefore, differs from the present invention which is drawn towards a method of treatment for anthracycline induced cardiotoxicity.

Therefore, in view of teachings of Barone et al that hydroxycarbazole derivatives being oxygen free radical scavengers or antioxidants, are useful for protection of the cardiovascular system from traumatic and post traumatic injury associated with myocardial infarction, as well as for prevention of oxidative tissue damage to organs afflicted with disease induced ischemic trauma, one skilled in the art can not be motivated to practice of the present invention viz. treatment and prevention of anthracycline induced cardiotoxicity etc. by administering 5-methoxytryptamine.

Moreover, contrary to what the Examiner suggests not all molecules which show antioxidant or free radical scavenging properties have cardioprotective action and are not in all probability useful for treatment of patients suffering from anthracycline induced cardiac toxicity, myocardial ischemia, myocardial infarction or heart failure.

For example, Vitamin E, which is a very well known antioxidant and used in a large number of pharmaceutical compositions to prevent oxidation, has been reported to be futile in preventing the cardiotoxicity or cardiac damage. Van Vleet et al (Am. J. of Pathol., **1980**, 99 13-24) has evaluated cardioprotective activity of Vitamin E and Selenium compounds in cardiac disease induced by chronic Adriamycin administration in dogs and has reported that parenteral administration of Vitamin E or Vitamin E-selenium concurrently with adriamycin treatment failed to alter the incidence and severity of cardiac damage present in dogs. The Examiner is invited to refer to lines 28-30 second paragraph, page number 21 of the article filed with this response. The authors have further reported that at the time of their work, the antioxidant activity of vitamin E as well as lipid peroxidation regulating properties of selenium in an endogenous system being a component of selenoenzyme, glutathione peroxidase was very well known (see lines 4-7, page number 22).

A similar report regarding failure of Vitamin E to protect against adriamycin induced Cardiotoxicity in rabbits was reported by Bredd et al (Cancer Research, **1980**, 40, 2033-2038), a copy of which is also enclosed herewith.

N-acetylcysteine (CAS Registry number 616-91-1), which is a well-known free-radical scavenger, has also been evaluated by Myers et al (Seminars in Oncology, **1983**, 10, Suppl. 1, 53-55) for its ability to prevent doxorubicin induced cardiomyopathy and they have reported that N-acetyl cysteine was not clearly

effective for the protection of doxorubicin induced cardiotoxicity in the dose and schedule used in this study. Similar results are reported by Herman et al. (Cancer Research, **1985**, 45, 276-281), who have also evaluated N-Acetylcysteine in preventing chronic doxorubicin induced cardiotoxicity in Beagles.

In addition, the failure of free radical scavengers or antioxidants to prevent doxorubicin indicated cardiotoxicity is discussed in the present application. Please refer to paragraphs number 0018 and 0019, page 2 of the published application US 2005/0020666, which are reproduced verbatim herein below:

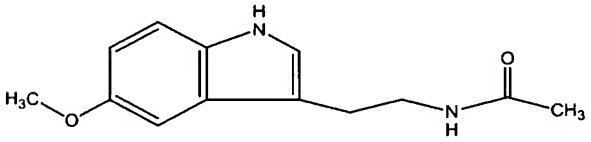
"[0018] Despite the Doxorubicin induced free radical formation (Yin, X. et al., Biochem Pharmacol, 1998,: 56: 87-93, Hershko, C. et al., Leuk. Lymphoma 1993, 11: 207-214) several of the well documented free radical scavengers and/or antioxidants do not protect against Doxorubicin induced cardiotoxicity in vivo. Studies with N-acetylcysteine or Vitamin E have shown that neither compound would prevent or significantly reduce cardiac lesions induced by chronic treatments with Doxorubicin (Herman, E. H. et al., Cancer Res., 1985, 45: 276-281 and van Vleet, J. F. et al., Am. J. Pathol, 1980, 99 :13-22, Breed, J. G. et al., Cancer Research, Vol 40, No. 6, 2033-2038, 1980).

[0019] Similar negative results were obtained in clinical trials in which patients were given Vitamin E (Legha, S. S. et al., Ann. N.Y. Acad Sci 1982, 393: 411-418) or N-acytlcysteine (Myers, C. et al., Semin. Oncol. 1983,10 (suppl) 53-55) prior to and/or concomitant with Doxorubicin".

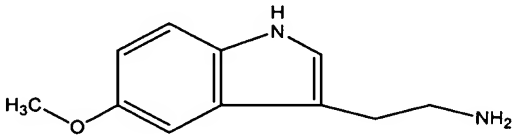
Thus from the above, it would be abundantly evident, that not all the antioxidants and free radical scavengers are effective in treating cardiotoxicity by virtue of their antioxidative and free-radical scavenging properties. To say in other words, antioxidative and free-radical scavenging properties could not be considered as "Gold Standard" for predicting the cardiotoxicity preventing potential of the agent.

While, the above discussion refers to molecules, which are structurally very distinct i.e. not related to the 5-methoxytryptamine, however, the same could also be said regarding molecules, which are structurally very closely related to 5-methoxytryptamine.

An analogue of 5-methoxytryptamine, Melatonin of formula –I, which differs from 5-methoxytryptamine of formula –II, only in possessing an additional acetyl functional group at amine nitrogen of ethylamine side chain, is also a well known free radical scavenger and an antioxidant.



Formula



Formula -II

Reiter, Russel J et al. in a review document (Journal of Pharmacy and Pharmacology, **2002**, 54, 1299-1321), while discussing drugs whose damaging effect is mediated by free radicals and related reactants, have reported that Melatonin protects the heart against the toxic reactions of doxorubicin, such as cardiomyopathy and congestive heart failure. Melatonin reduces doxorubicin induced lipid peroxidation and lactate dehydrogenase leakage in cardiomyocytes, thereby reduces the side effects of doxorubicin and epirubicin. (A copy of this article was filed with the Information Disclosure Statement dated September 16, 2004).

However, all the prior-art documents related to melatonin emphasizes that the antioxidant, and cholesterol lowering (lipid peroxidation inhibition) properties are mainly responsible for observed cardioprotective effect.

As discussed in this application, (please see paragraphs [0010] and [0011] of the published specification) the complete and exact mechanisms for doxorubicin and other anthracycline induced cardiotoxicity are not completely understood. There exists no single unified mechanism of cardiotoxicity of anthracyclines. The mechanism by which anthracyclines exert their cytotoxic activity is complex, involving multiple intracellular effects. Free radical formation is involved in both the antineoplastic and toxic effects of these drugs. Further, proposed mechanisms include inhibition of specific enzymes, changes in cardiac muscle expression, alteration in myocardial membrane function, sensitization of calcium release from sarcoplasmic reticulumin and mitochondrial DNA damage and induction in apoptosis.

Further, it might be noted that there are several enzyme markers as well as serum markers indicative of tissue injury / ischemia/ stress/hypoxia. These include enzymes such as creatinine kinase, and its myocardium specific isoform i.e. creatinine kinase – MB (CK-MB), lactate dehydrogenase, superoxide dismutase, and membrane lipid peroxidation. The activity of these markers is a well accepted index of cardiac toxicity and could be used to develop a cardioprotective agent. CK-MB is one such marker and currently is the only one accepted by and recommended in the World Health Organization (WHO) Guidelines for the diagnosis of acute myocardial infarction and has become the "Gold Standard" for assessing myocardial function (MI).

In view of the above information, the applicants surprisingly found that 5-methoxy tryptamine lowers, the Gold Standard, CK-MB levels and could be used as an efficient cardioprotective agent in anthracycline induced cardiotoxicity as anthracycline treatment produces significant elevation in CK-MB levels.

As of today, the applicants have neither come across any literature, wherein the effect of melatonin on circulating levels of CK-MB was observed. Since CK-MB

being a gold standard for assessing myocardial function definitely has a major, predictable and reliable role. Thus, the available information of antioxidative or cholesterol lowering properties of melatonin does not provide any motivation to a person to try the analogues of melatonin for evaluating the lowering effect on CK-MB levels. Nor does such disclosure provide one of skill in the art with a reasonable expectation of success.

Further, it is the applicants' finding that melatonin does not reduce cardiac injury caused by doxorubicin administration, whereas 5-MT does reduce the cardiotoxicity, when evaluated in controlled acute cardiotoxictiy models in terms of circulating CK-MB values, which is a 'Gold Standard' for assessing myocardial infaraction. This would be evident from the comparative data summarized in Table-I, below wherein the effect of both melatonin and 5-MT on doxorubicin induced cardiotoxicity is evaluated in terms of circulating level of creatinine kinase-MB.

In the study, which was performed to evaluate the effect of both melatonin and 5-MT on doxorubicin induced cardiotoxicity, each group consisted of six animals. Melatonin and 5-MT were administered to rats before administration of adriamycin, a known cardiotoxicity inducer. Adriamycin (20 mg/kg) was administered to animals of Group II to Group VIII. The rats in the groups III ,IV and V were treated with melatonin at a dose of 17.5 mg/kg to 70 mg/kg, whereas the rats in the groups VI, VII and VIII received 5 MT in a dose of 17.5mg/kg to 70 mg/kg. Both Melatonin and 5 MT were given orally

Table-I: Effect of Melatonin and 5-MT on Adriamycin Induced Cardiotoxicity

Sr. No.	Groups	CK-MB ± SEM (U/L)
I	Control	3220 ± 280.6
II	Adriamycin (20.0 mg/kg)	4295 ± 341.3
III	Melatonin (17.5 mg/kg) + Adriamycin (20.0 mg/kg)	4965 ± 134.4

IV	Melatonin (35.0 mg/kg) + Adriamycin (20.0 mg/kg)	4470 ± 159.8
V	Melatonin (70.0 mg/kg) + Adriamycin (20.0 mg/kg)	4450 ± 261.4
VI	5-MT (17.5 mg/kg) + Adriamycin (20.0 mg/kg)	3625 ± 119.7
VII	5-MT (35.0 mg/kg) + Adriamycin (20.0 mg/kg)	3605 ± 111.9
VIII	5-MT (70.0 mg/kg) + Adriamycin (20.0 mg/kg)	3332 ± 120.5

From Table –I, it would be evident that when rats were treated with adriamycin (Group -II), there is a 33% increase in the CK-MB levels in comparison to control rats (Group –I), which is indicative of myocardial tissues injury. Melatonin at various doses (17.5 mg/kg, 35 mg/kg and 70 mg/kg) does not produce any reduction in the circulating CK-MB values of adriamycin treated rats, whereas there was approximately 15 – 22 % decrease in the circulating CK – MB values when the animals were treated with 5-MT in the same range of concentration.

Thus, melatonin does not produce any protective effect in terms of circulating CK-MB values in the animals treated with Adriamycin, whereas 5-MT lowers the circulating CK-MB values and therefore, could be used clinically to prevent and/or treat the cardiotoxicity associated with anthracycline glycosides.

Since, 5-methoxy tryptamine, an analogue of melatonin, acts through Gold Standard, CK-MB, whereas melatonin does not show any reducing effect in CK-MB level, the cardioprotective effect observed with 5-methoxy tryptamine has to be of different amplitude than that observed with melatonin and therefore, the reported data of cardioprotective activity of melatonin could not be extrapolated to determine the cardioprotective effect of 5-methoxy tryptamine.

The standard test used to establish *prima facie* obviousness is the test set out by the Supreme Court in *Graham v. John Deere* (383 US 1, 148 USPQ 459 (1966)). To determine whether a claim is *prima facie* obvious:

1) the scope and content of the prior art are to be determined;

2) the differences between the prior art and the claims at issue are to be ascertained; and

3) the level of ordinary skill in the pertinent art resolved.

In addition, according to MPEP 2141, citing *Hodosh v. Block Drug Co., Inc.,* 786 F.2d 1136, 1143 n.5, 229 USPQ 182, 187 n. 5 (Fed. Cir. 1986), when applying 35 USC 103, the following tenets of patent law must be adhered to:

1) the claimed invention must be considered as a whole;

2) the references must be considered as a whole and must suggest the desirability and thus the obviousness of making the combination; and

3) the references must be viewed without the benefit of impermissible hindsight vision afforded by the claimed invention.

Reasonable expectation of success is the standard with which obviousness is determined. *In re Merck & Co., Inc.*, 800 F.2d109, 231 USPQ 375 (Fed. Cir. 1986).

The reason, suggestion or motivation to combine references may be found explicitly or implicitly. While the references need not expressly teach that the disclosure contained therein should be combined with another, the showing of combinability must be clear and particular. *Ruiz v. A.B. Chance Co.,* 57 USPQ2d

1161 (Fed. Cir. 2000).

In looking at the prior art that may be used to assess obviousness, there is a presumption that the skilled worker knows of everything that has been published, publicly used etc. *In re Winslow*, 365 F. 2d. 1017, 151 USPQ 48 (CCPA 1966). (As the court put it, "We think the proper way to apply the 103 obviousness test to a case like this is to first picture the inventor as working in his shop with the prior art references-which he is presumed to know-hanging on the walls around him...Section 103 requires us to presume full knowledge by the inventor of the prior art in the field of his endeavor").

Therefore, in view of the discussion above concerning the scope and content of the prior art of Liu et al. and Barone et al.; the differences between the prior art and the claims at issue the level of ordinary skill in the pertinent art and the fact that that references considered as a whole do not suggest the desirability and thus the obviousness of making the combination; it is clear that claims 16, 18, 21, 24 and 29-43 are not obvious.

Therefore, it is respectfully requested that the rejection be withdrawn.

It is submitted that the present application is in condition for allowance and favorable consideration is respectfully

Respectfully submitted,

Janet I. Cord
Ladas & Parry LLP
26 W. 61st Street
New York, NY 10026
Reg. No. 33,778
Tel. No.: (212)708-1935